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Genetic Knowledge-based Artificial Control over Neurogenesis in Human Cells Using Synthetic Transcription Factor Mimics(Abstract_要旨)

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| 論文題目 | Genetic Knowledge-based Artificial Control over Neurogenesis in Human Cells Using Synthetic Transcription Factor Mimics (転写因子を模倣した合成分子による、遺伝子塩基配列情報に基づく神経発生制御に関する研究) | | |
| (論文内容の要旨) | | | |
| <p>The epigenetic mechanism controls gene expression without alteration in DNA sequence. Chromatin structure changes the histone affinity to the related DNA and also alters the accessibility of transcription factors and RNA polymerase packages to chromatin. Through this 3-D structural modification of chromosome, gene transcription operates in between activation or repression.</p> <p>The central epigenetic control mechanism includes DNA methylation, histone modification by methylation or acetylation, chromatin remodeling and interference by non-coding RNAs. Epigenetic function in the formation of neural cells especially the role of histone modification in neural differentiation has also been well clarified. The epigenetic enzymes histone acetyltransferase (HAT) and histone deacetylase (HDAC) operate in tandem to decide the transcription status of the histones. Therefore, controlling the acetylation or de acetylation of local chromatin architecture of key genes in neural processes may contribute to gaining control over the neural differentiation. Our designer gene ON or OFF switching small molecules SAHA-L and PIP-RBPJ-1 operate by inhibiting HDAC or by blocking the transcription factor in the promoter or enhancer region of the target gene. Consequently, these molecules have the ability in editing epigenetic status of a particular gene that controls the cell differentiation in neural systems. Harnessing SAHA-L, we successfully enhanced the differentiation of the iPSCs into primary neural cells. Likewise, using PIP-RBPJ-1 that operate by blocking the RBPJ protein' s binding in Hes1 promoter region, we gained chemical control over the Notch signaling and increased the neuronal differentiation rate to 68.4% compared with the control. Besides in recent years, genetic-knowledge based medicine has been proclaimed to be useful in developing therapeutic strategies to treat incurable neural disorders. However, there is a lack of information about the control factors in rare neural diseases like ATRX that is known to occur by gene mutation of the ATPase related SNF2 chromatin remodeling in H3.3. To this end, we carried out studies and gained information about the key genetic and epigenetic actors in the mutated ATRX-iPS cell lines and in the neural cells derived from them.</p> <p>1. SAHA-L could manuscript therapeutically Significant Nervous system Genes</p> | | | |

and enhance neural induction from iPS cells

An integrated multitarget small molecule capable of altering dynamic epigenetic and transcription programs associated with the brain and nervous system has versatile applications in the regulation of therapeutic and cell fate genes. Recently, we have been constructing targeting epigenetic ON switches by integrating sequence-specific DNA-binding pyrrole-imidazole polyamides with a potent histone deacetylase (HDAC) inhibitor SAHA. Using microarray studies, here we report on the identification of a DNA-based epigenetic ON switch, termed SAHA-L, as the first multitarget small molecule capable of inducing transcription programs associated with the human nervous system and brain synapse networks in BJ human foreskin fibroblasts and 201B7-iPS cells. Ingenuity Pathway Analysis showed that SAHA-L activates the signaling of synaptic receptors such as glutamate and γ -aminobutyric acid, which are key components in autism spectrum disorders. Real-time PCR showed that SAHA-L could significantly ($p < 0.05$) induce the expression of brain- and nervous system-associated REELIN, CNTNAP2, NOS1AP and KALIRIN. Prolonged incubation of 201B7-iPS cells with SAHA-L caused changes in the morphology and shifted the transcriptional program from a pluripotent state to a neural progenitor state. We also used immunostaining to confirm the generation of NESTIN-positive cells. Our findings suggest that tunable SAHA-L has potential as a cell-type-independent multitarget small molecule for therapeutic and/or cell fate gene modulation.

2. A Synthetic DNA-binding inhibitor of HES1 alters the Notch signaling pathway and induces neuronal differentiation

Synthetic DNA-binding inhibitors of cell fate-regulating transcription factors are of increasing demand because they could precisely alter the complex transcription machinery associated with cell fate specification. Basic-helix-loop-helix transcription factors like hairy and enhancer of split 1 (HES1) operate as the negative regulator of neuronal differentiation and govern the transcription program associated with neural stem cells (NSC). To gain chemical control over NSC differentiation, we harnessed the sequence information and designed pyrrole-imidazole polyamides (PIPs) as DNA-binding inhibitors of HES1 targeting RBPJ, which is a key transcription regulator of neural development-governing notch signaling pathway. Biological evaluation studies demonstrated the capability of a DNA-binding inhibitor of RBPJ called PIP-RBPJ-1 to inhibit the endogenous expression of HES1 and ensue the induction of neuronal markers ASCL1 and NGN2. Genome-wide gene expression studies revealed that PIP-RBPJ1 also regulates notch-signaling pathway and shift the transcription program

from pluripotency to neural state. Also, PIP-RBPJ-1 successfully generated TUJ active neurons with longer neurite outgrowth with an efficiency that is comparable to the conventional protocol to suggest the potential of DNA-binding inhibitor to induce targeted differentiation

3. ATRX patient HDF cell derived iPS have different character in the neuron differentiation

ATRX gene, underlie a number of genetic disorders including several X-linked mental retardation syndromes, which encoding chromatin-remodeling proteins. However, the role of ATRX protein in normal neurogenesis *in vitro* is unknown. Here, we used an ATRX patient derived ATRX gene single site mutation iPS cell line A196, and A197 to clarify the mutated gene related neural characters *in vitro*. Mutation of ATRX patient derived protein caused notable widespread differences in the following stages. In the neural induction stage, Atrx patient-derived iPS generated fewer neural stem cells; In the differentiation stage, ATRX patient derived cells was observed to have relatively more number of glial cells than that of neurons. In the mature stage, ATRX neurons did not have the capability to generate functional neurons with physiological properties. Also, the GABA content was less compared with the normal neurons, which has also been proved by microarray analysis, and PCP contributes a lot to these difference. Taken together, our results indicate that ATRX is a critical mediator during early neuronal differentiation. Thus, the increased neuronal loss may contribute to the severe mental retardation observed in human ATRX patients. This in vitro disease modal may give biological insight and aids in combining the basic research to the clinical research to gain genetic-knowledge based artificial control over neural cell fate.

(論文審査の結果の要旨)

任意の遺伝子発現を人為的にコントロールする遺伝子スイッチの開発は細胞の初期化、iPS 細胞の分化、新しい病気の治療法として注目されている。DNA に塩基配列選択的に DNA に結合するピロールーイミダゾールポリアミド(PIP)は遺伝子スイッチの骨格として注目を集めており、我々の研究グループでは様々な PIP を用いて遺伝子発現の制御について検討している。神経疾患は高齢化社会において患者数も多く社会的な問題になっているが、その有効な治療法はほとんどない。

今回、申請者は PIP 誘導体を用いて神経系遺伝子の発現の制御について分子レベルで検討を行った。まずはじめにマイクロアレーを用いた遺伝子の発現解析からスクリーニングされた HDAC 阻害剤 PIP コンジュゲートである SAHA-L を用いて神経幹細胞特有の遺伝子の活性化を検討した。その結果、繊維芽細胞や iPS 細胞に SAHA-L を作用させると REELIN、CNTNAP2、NOS1AP、KALIRIN などの神経関連遺伝子の活性化が効率よく起こることが示された。

次に神経幹細胞を用いてニューロンへの分化誘導について検討した。これまでの研究から Hes1 遺伝子の発現を抑えることによりニューロンへの分化が期待されたので、Hes1 遺伝子のプロモーターに存在する RBPJ 結合サイトに結合する PIP-RBPJ-1 を設計した。PIP-RBPJ-1 を神経幹細胞に作用させるとマーカー遺伝子である ASCL1 や NGN2 の発現がみられ、長期培養することによって TUJ を発現するニューロンに分化誘導できることに成功した。

また神経疾患のモデルとして ATRX について検討を行った。ATRX は X 染色体上の ATRX 遺伝子の変異によって神経遅滞と貧血がおこる遺伝性疾患である。ATRX の患者細胞から作成した 2 種の iPS 細胞を常法にしたがってニューロンに分化させた。その結果 ATRX の患者由来の iPS 細胞からは神経幹細胞の形成効率が低く、また電気生理学的に機能があるニューロンが形成しないことが明らかになった。

塩基配列選択的に DNA に結合する PIP を用いた神経幹細胞への分化誘導や、神経幹細胞からニューロンへの分化は初めての例であり、独創的な研究と言える。またこれまで不明であった ATRX について発症のメカニズムにも研究の糸口を与えた。よって、本論文は博士(理学)の学位論文として価値あるものと認められる。また、平成 30 年 1 月 16 日、論文内容とそれに関連した事項について試問を行った結果、合格と認めた。